DECODING RESISTANT HYPERTENSION SIGNALING PATHWAYS

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Abstract Resistant Hypertension (RH) is a clinical condition in which the hypertensive patient has become resistant to drug therapy and is often associated with increased cardiovascular morbidity and mortality. Several signaling pathways have been studied and related to the development and progression of RH: Modulation of sympathetic activity by leptin and aldosterone, primary aldosteronism, arterial stiffness, endothelial dysfunction and variations in the renin-angiotensin-aldosterone system (RAAS). MicroRNAs (miRNA) comprise a family of small noncoding RNAs that participate in the regulation of gene expression at post-transcriptional level. miRNAs are involved in the development of both cardiovascular damage and hypertension. Little is known on the molecular mechanisms that lead to development and progression of this condition. This review aims to cover the potential roles of microRNAs in the mechanisms associated with the development and consequences of RH, and explore the current state of the art of diagnostic and therapeutic tools based on microRNA approaches.

Keywords MicroRNA**;** Resistant Hypertension**;** Signaling Pathways

Abbreviations List

20-HETE - 20-hydroxyeicosatetraenoic acid

ABPM - ambulatory blood pressure monitoring

ACE - angiotensin converting enzyme

ADMA - asymmetric dimethylarginine

AGT - angiotensinogen

ALLHAT - antihypertensive and lipid-lowering treatment to prevent heart attack trial

AngI - angiotensin I

AngII - angiotensin II

APCs - angiogenic progenitor cells

AT1 - angiotensin 1 receptor

AVPR1A - arginine-vasopressin receptor

Bcl-2 - B-cell lymphoma 2

BDKRB2 - bradykinin β2 receptor

bFGF - basic fibroblast growth factor

BP - blood pressure

CAV1 - Caveolin-1

COX-2 - cyclooxygenase-2

CYP4A - cytochrome P450-4A)

EGF - epidermal growth factor

EGFR - Epidermal growth factor receptor

eNOS - endothelial NO synthase

ERK - extracellular regulated kinase

ET-1 - endothelin-1

FOXO1 - forkhead box 1

FoxO1 - forkhead Transcription factor

GATA2 - endothelial transcription factor GATA2

GLUT4- glucose transporter 4

GPCRs - G-protein-coupled receptors

HDL - High density lipoprotein cholesterol

LNA - locked-nucleic-acid

MAPKs - mitogen-activated protein kinases

MCP-1 - monocyte chemoattractant protein-1

MEK - mitogen-activated protein kinase

miRNA – microRNAs

MMP - matrix metalloproteinase

mTOR - mammalian target of repamycin

NO - nitric oxide

NYHA - New York Heart Association

PAK4 - serine/threonine-protein kinase PAK4

PDGF - platelet-derived growth factor

PG - prostaglandins

PI3K - phosphoinositide 3-kinase

PIK3R2 - phosphoinositide-3-kinase regulatory subunit 2

PIP3 - phosphatidylinositol 3,4,5-trisphosphate

PKC - protein kinase C

PLC - phospholipase C

PLD - phospholipase D

pri-miRNA - primary miRNAs

RAAS - renin-angiotensin-aldosterone system

RH - resistant hypertension

RhoB - ras homolog family member B

RISC - RNA-induced silencing complex

ROS - reactive oxygen species

RTKs - receptor tyrosine kinases

S1P - sphingosine-1-phosphate

SirT1 - silent information regulator 1

SNS - Sympathetic nervous system

Spred-1 - Sprouty-related protein

SRF - Serum Response Fator

TBXA2R - thromboxane A2 receptor

VCAM1 - vascular cell adhesion molecule 1

VEGF - vascular endothelial growth fator

VSMC - vascular smooth muscle cells

1.Introduction

Resistant Hypertension

Resistant hypertension (RH) is characterized by a condition in which the patient requires four or more antihypertensive medications, including a diuretic, regardless blood pressure control. RH patients can be classified as controlled or uncontrolled according to the achievement of the blood pressure goals (1).

The RH affects approximately 13% to 25% of the hypertensive population (2-4)and represents a risk factor for cardiovascular events. Results from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) showed increased hazard ratios for stroke (1.57), end-stage renal disease (1.95), heart failure (1.88), coronary heart disease (1.44) and all-cause mortality (1.30) in RH compared to non-resistant hypertensive subjects(5).

RH is a multifactorial condition and several environmental and genetic factors contribute to the development and progression of the disease. Firstly, the identification of pseudoresistence must be performed to identify true resistant hypertensive patients, caused mainly due to poor BP measurements, lack of adherence, suboptimal therapy and white coat hypertension. In addition, secondary causes of RH, such as primary aldosteronism, pheochromocytoma, renal artery stenosis or Cushing's syndrome should be identified since the pharmacological treatment will be specific for each condition. Among the factors associated with an increased risk for RH are: older age, African origin, female gender, overweight and obesity (1, 6).

The RH present three relevant characteristics: 1) high incidence of the following comorbidities: obstructive sleep apnea (7), thyroid disorders (7), primary aldosteronism (8, 9), reduced plasmatic renin activity, obesity (6), diabetes *mellitus* (1, 6) 2) high prevalence of target organ damage; and 3) high blood pressure (BP) levels measured by ambulatory blood pressure monitoring (ABPM) (10-12).

The achievement of the BP goals relies on physician examination and on patient characteristics and compliance to pharmacological and non-pharmacological treatments. It is well know that obesity, excessive alcohol and/or salt intake, sedentary lifestyle, smoking, insulin resistance, difficulty in adopting dietary measures and lack of adherence to therapeutic treatment affects BP control (12). In addition, prescription of

high-cost medicines by physicians, multiple administration regimens, suboptimal doses and presence of adverse effects are associated with uncontrolled BP (13).

The factors associated with diagnostic and treatment of RH, including lifestyle, detailed history of medication adherence, correct BP measurement, biochemical analysis for dosage of electrolytes, glucose and creatinine, as well as determination of protein and sodium in the urine (12).

It is also necessary the exclusion of pseudohypertension. For example, Mönckeberg's sclerosis is a condition characterized by the loss of elasticity and thickening of the walls of the muscular arteries caused by a calcification of the tunica media constituted of smooth muscle. In the measurement of blood pressure by non-invasive methods, the patient presents high BP values, while in reality it pressure is normal, therefore, it is necessary to use invasive measurement methods to correctly measure it BP (12). White coat hypertension, also a condition of pseudoresistance, where the patient exhibits high values during the verification in the physician's office. It can be excluded by 24 hours ABPM. It is estimated that 30% of patients with elevated BP and with treatment of up to 3 drugs present this condition of white coat hypertension (12, 14).

In order to complete the diagnosis of RH, there are some clinical situations that are considered as secondary causes of this condition, such as: primary hyperaldosteronism, pheochromocytoma, fibromuscular dysplasia, patients with increased risk of atherogenesis (12), obstructive sleep apnea, renal artery stenosis, renal parenchymal disease, Cushing's syndrome, and thyroid and parathyroid diseases (10). These forms of secondary hypertension present high prevalence in association with RH, as can be verified in table 1.Hyperaldosteronism results from the excessive production of aldosterone, a hormone that is produced in the adrenal glands and that decreases the excretion of sodium and increase the excretion of potassium by the kidneys, sweating, and saliva. The determination of the rate of aldosterone/renin ratio is used as screening tool for hyperaldosteronism diagnostic, and if any alteration is confirmed, diagnostic imaging and blood samples from each side of the adrenal glands are used to corroborate the diagnosis (12).Obstructive sleep apnea consists of the collapsing of the pharynx walls hampering the adequate respiration of the individual. The patient is then submitted to a nocturnal polysomnography in order to monitor respiration and body functions

duringsleep for diagnostic of the condition (15). For more information on diagnostic and treatment methods, see table 1 (14) .

A flowchart (**Figure** 1) summarizes the steps involved in the diagnostic of RH according to the American Heart Association Statement (1, 16).

[TABLE 1]

[FIGURE 1]

Although RH is a multifactorial condition, sodium excess, fluid retention, increased activation of the Renin Angiotensin Aldosterone System and higher sympathetic tone are among the most well-described mechanisms of BP elevation in RH. The complex pathophysiology of the development and progression of RH requires further investigation to identify molecular mechanisms that could be translated into diagnostic and more assertive therapeutic strategies. In the following sessions, the signaling pathways and the participation of miRNA in their regulation will be discussed.

2. Renin Angiotensin Aldosterone System

The Renin-Angiotensin-Aldosterone System (RAAS) is responsible for the hemodynamic equilibrium. This is possible due to the effects of this system on the kidneys, that act in the sodium water balance, and also due to the influence in the vascular resistance on the peripheral blood vessels, thus permitting the maintenance of the BP (17). In order to the RAAS to produce a response it is necessary that occur some type of alteration in the circulating blood volume, such as blood loss, dehydration or even pumping failure by the ventricles (18) (**Figure** 2).

Juxtaglomerular apparatus comprises afferent arterioles in the distal part of the ascending branch of the loop of Henle in the renal glomeruli. The cells that line these arterioles in the region of the apparatus are called juxtaglomerular cells and are able to recognize the BP inside these vessels (18). Moreover, the cells that line the loop of Henle in the region of the juxtaglomerular apparatus are called macula densa and respond to changes in the sodium concentration of the filtrate. By detecting these changes, the cells of the dense macula stimulate juxtaglomerular cells to produce an enzyme called renin that is released into the bloodstream. This enzyme is responsible for the production of angiotensin I (AngI) through the cleavage of angiotensinogen, which is synthesized and secreted by the liver. In the pulmonary and renal endothelium, an enzyme called angiotensin converting enzyme (ACE), which hydrolyzes the circulating AngI in angiotensin II (AngII), is present. The angiotensin 1 receptor (AT1) is activated by AngII thus promoting a vasoconstrictive response in the blood vessels, in addition to stimulating the adrenal gland to produce aldosterone. The renal tubules respond to the aldosterone by retention of sodium and water, which will promote increased blood volume and consequently, BP elevation (19, 20).

[**FIGURE** 2]

The RAAS constitutes the main signaling pathway involved in the long-term control of the BP. Innumerous regulators participate in this biochemical cascade of communication such as renin, angiotensinogen, AngI and II, ACE 1 and 2, aldosterone and angiotensin- (1-7) [Ang- (1-7)]. The deregulation of one or more effectors of this system contributes to failures in blood pressure control, usually leading to hypertension (21). Resistant Hypertension is accompanied by intravascular fluid retention that can be attributed, at least in part, to dysregulation in the renin-angiotensin-aldosterone system. Previous studies have found evidence of intravascular volume expansion (higher levels of brain-type natriuretic peptide – BNP, and atrial natriuretic peptide - ANP) and aldosterone excess (higher levels of plasma and urinary aldosterone, aldosterone to renin ratio) in resistant hypertension compared to controls (22). Similarly, another study reported higher volume of fluid by thoracic electrical bioimpedance, sugesting that the intensificaton of diuretic thereapy in those patients could be beneficial (23). ANP and BNP are hormones that regulate cardiovascular hemodynamics. They are secreted by, respectively, cardiac atria and cardiac ventricles, in response to stretch or pressure. Natriuretic effects are mediate by subtype A-natriuretic peptide receptor, which is expressed in several tissues, including kidneys, blood vessels, adrenal glands, and adipose tissue. ANP produces its natriuretic actions by increases glomerular filtration rate, inhibits sodium transport in proximal tubule and inhibition of aldosterone release in adrenal cells. The latter effect is also attributed to BNP (24). Aldosterone is one of the most studied RAAS components in RH, several studies had shown that aldosterone excess is a common characteristic of RH. In addition, primary aldosteronism is the most common secondary cause in the patients with RH (8). This condition is characterized by excessive autonomic secretion of aldosterone by the adrenal gland, being the production of adenomas and idiopathic hyperaldosteronism the main forms (32, 33). This secretion, stimulated by renin, promotes the retention of sodium and water, promoting the elevation of blood volume, and consequently the increase of BP. When it is released into the bloodstream, the aldosterone diffuses through the membrane into the cytosol of renal tubular epithelial cells, subsequently binding to a family of NRC2-type mineralocorticoid receptors. This aldosterone-receptor complex will be translocated to

the nucleus, activating the synthesis of proteins related to sodium and potassium transport, such as Na^+ K^+ ATPase (**Figure** 3) (25).

[**FIGURE** 3]

An important signaling pathway in the primary aldosteronism is the PI3K (phosphoinositide 3-kinase) pathway with the activation of mTOR (mammalian target of repamycin), when overactivated is involved in the tumorigenesis and metastasis in some types of human tumors such as renal cancer, adrenal carcinoma and pheochromocytoma (26-28) (**Figure** 4) (27). The PI3K/AKT/mTOR pathway is regulated in response to the signaling of growth factors such as the epidermal growth factor (EGF) through receptor tyrosine kinases (RTKs)(29).

Another stimulus to the release of aldosterone is the action of sphingosine-1-phosphate (S1P) by means of the activation of the PI3K/AKT (protein kinase B) and MEK (mitogen-activated protein kinase)/ERK (extracellular regulated kinase) pathway in glomerular cells of the adrenal glands (**Figure** 4) (28). S1P is a bioactive sphingolipid intracellularly formed that acts as a second messenger mediating regulatory processes such as cell differentiation, migration and contraction, modulation of immune response and angiogenesis, and this molecule is considered to be the key hormone for hemodynamic stability in humans (30, 31). Its action involves the activation of phospholipase D (PLD), calcium influx (Ca^{2+}) from the extracellular medium and phosphorylation of α and β isoforms of protein kinase C (PKC) (30, 32, 33).

[**FIGURE** 4]

Previous studies had shown excess of aldosterone in uncontrolled RH compared to controlled group. The same study demonstrated that aldosterone was correlated to arterial stiffness (34). Furthermore, higher aldosterone levels were associated with the T allele for the polymorphism -344 C/T *CYP11B2* (aldosterone synthase gene) in RH

subjects, and this effect was shown to be more pronounced in patients under spironolactone treatment (35). Studies have demonstrated significant reductions in blood pressure with addition of mineralocorticoid receptors antagonists, such as spironolactone, and that drug has been suggested as the optimal fourth-line drug for BP control in RH (159).

Angio-(1-7) is a heptapeptide that carries out an important function in the RAAS. This molecule is formed both by the action of ACE 1 (dependent pathway) and the hydrolysis of AngII by the ACE 2 (independent pathway) (36, 37) being this last one the most important pathway in the formation of Ang-(1-7) (38). This molecule produces its AngII endogenous counter-regulatory effects on RAAS (vasodilation, cardio protection, natriuresis and diuresis, angiogenesis inhibition, and cellular growth) (39) through the binding to its specific receptor called Mas, a G protein-coupled receptor (32, 40, 41).

The ACE2/Ang-(1-7)/Mas signaling pathway consists in one of the RAAS axes that opposes, in terms of function, to another classical axis of this system, the ACE/AngII/AT_1R. The imbalance of these two opposing axes, mainly in the direction of the ACE/AngII/AT_1R axis predisposes to cardiovascular diseases and other disorders (37, 41).

The Ang-(1-7)/Mas complex regulates different signaling pathways, such as PI3K/AKT and ERK signal, and involves the maintenance of some effectors like nitric oxide (NO)(32, 41), FOXO1 (forkhead box 1)(39) and cyclooxygenase-2 (COX-2)(40) (**Figure** 5). Studies report that due to the participation of the Ang-(1-7) in these mechanisms, this heptapeptide is related to pathological conditions such as fibrosis and inflammatory processes in some organs, like lungs, liver and kidneys (42). Other findings demonstrate that Ang-(1-7), through the interaction with its Mas receptor, stimulates the activation of the nitric oxide synthesis (eNOS) in endothelial cells, promoting vasodilation (32, 36, 41).

Another study demonstrates that Ang-(1-7), through the interaction with its specific Mas receptor promotes the increase of nitric oxide (NO) and prostaglandins (PG) synthesis and release leading to vasodilation and inhibition of cellular growth, opposing to the vasoconstrictor and proliferative effects mediated by the interaction of AngII with its AT_1 receptors. The imbalance between these two axes of the RAAS,

reflected by the imbalance between theses peptides, which are observed in cardiovascular diseases, can lead to the decrease of NO and consequently to endothelial dysfunction (**Figure** 5) (43).

[**FIGURE** 5]

2.3. Sympathetic Nervous System

Sympathetic nervous system regulates cardiac output and peripheral vascular resistance (vasoconstriction) through release of norepinephrine and epinephrine, resulting in increase in blood pressure. At the renal level, SNS activation increases renin release from juxtaglomerular cells and modulate tubular sodium reabsorption (44). RH patients have reduced heart rate variability, which is a marker for SNS activity. It was shown that 63% of the patients present a nondipping pattern (BP do not drop at night), which indicate sympathetic overflow. Moreover, sympathetic activation also increase sodium reabsorption and promote renin secretion and renal denervation has been investigated for treatment of resistant hypertension. In spite first studies in humans had shown promising results, randomized and blinded clinical trials demonstrated no benefit on BP control compared to sham procedure (45). Another intervention that has been tested is baroreflex activation therapy. Carotid sinus stimulation reduces BP in patients with uncontrolled RH, showing the import role of sympathetic activity in this condition (46-48).

2.4 - Adipokines

Adiponectin and leptin are two of the adipokines produced in adipose tissue. Obesity is an important comorbidity in RH and plasma levels of adiponectin and leptin were reported to be lower and higher, respectively in RH (34) (49). Leptin is a peptide hormone that is expressed in a variety of tissues, such as lymphoid tissue, pituitary gland, skeletal muscle, placenta, and ovary (50). However, white adipose tissue is the main responsible for the synthesis and secretion of this peptide, which has as effects to act on the hypothalamus in order to decrease appetite and stimulate sympathetic activity of the nervous system (**Figure** 6) (51). Elevated levels of leptin stimulate neurons in the hypothalamus to secrete a precursor protein that is cleaved in α-melanocyte stimulating hormone, which binds to melanocortin 3 and melanocortin 4 receptors. The binding of this peptide to the receptors stimulates the sympathetic nervous system, elevates the energy expenditure, decreases food intake, and activates the hypothalamic-pituitary-adrenal axis (52-54). A mechanism that demonstrates which factors are involved in the generation of hypertension associated with obesity is represented below (**Figure** 6) (51).

[**FIGURE** 6]

2.5. Insulin resistance and hypertension: the role of the Caveolin-1 (*CAV1*) gene

It was recently demonstrated that the gene Caveolin-1 (*CAV1*), located in the chromosome 7q31.1 (55) constitutes a gene that is associated with metabolic dysfunction in animal and cellular models, especially in insulin resistance, proving to be a potential marker for this condition in human beings (56). Genetic variations in *CAV1* are involved in the mechanism of insulin signaling and vascular function (**Figure** 7), shown in studies with animal models and cell culture (57, 58).

Increase *in* homeostasis model assessment *of* insulin resistance *(HOMA-IR)*, showing that *CAV1* is not only a genetic marker for dysfunction but also provides information about a potential mechanism of development of insulin resistance and hypertension in humans (56).

CAV1 is a regulatory gene for insulin signaling and insulin receptor stability (56). Specifically, *CAV1* binds directly to the insulin receptor in the adipocytes and the disturbance of this complex by GM3 ganglioside causes alteration in insulin signaling

(59) (**Figures** 7 and 8). In addition, the decrease in *CAV1* activity results in a 90% reduction in insulin receptor levels in the adipocytes of knockout rats (60).

Although the role of *CAV1* in insulin-mediated glucose uptake is not well elucidated (60), this gene demonstrated relevance in the translocation of glucose transporter 4 (GLUT4) to adipocyte (61) and muscle cells (62).

[**FIGURE** 7]

[**FIGURE** 8]

2.6 Vascular stiffness and endothelial dysfunction

Previous studies has been showed the participation of vascular stiffness and endothelial dysfunction in the pathogenesis of resistant hypertension. An increased carotid-femoral pulse wave velocity was observed in RH patients compared to non RH hypertension, demonstrating the impairment of elasticity in these vessels. In addition, flow-mediated dilation was found to be reduced in RH, reflecting an endothelial dysfunction (63).

2.6.1 Epidermal growth factor receptor in the vascular smooth muscle

The activation of the signaling pathway of the EGFR by matrix metalloproteinase (MMP), stimulated by GPCR agonists, such as catecholamines, endothelin-1 (ET-1) and AngII lead to the increase of the oxidative stress, promote stimulation of the hypertrophic growth and consequently increase of the muscular tone in hypertension (**Figure** 9) (64). Among these receptors it can be mentioned adrenoceptors and angiotensin receptors that contribute to the hypertension pathogenesis mainly through the vasoconstrictor effects produced after stimulation (65, 66).

The vasoconstrictor responses promoted by this pathway are mediated by phospholipase C (PLC), DAG and Ca^{2+} besides the growth promotion pathway

involving the tyrosine receptor and mitogen-activated protein kinases (MAPKs) (**Figure** 9) (67). Studies have shown a connection between the GPCR stimulus with the MAPK signaling pathway (through the dependent activation of MPM) in the vascular smooth muscle cells (65, 68-70). Associations have been made between GPCR stimulus by MPM's such as MPM-2, MPM-3, and MPM-7 in cardiomyocytes, fibroblasts and epithelial and endothelial cells (71) with consequent development of cardiovascular hypertrophy associated with hypertension (72-75).

[**FIGURE** 9]

2.6.2 CYP4A (cytochrome P450-4A)/20-HETE (20-hydroxyeicosatetraenoic acid)

20-HETE is an arachidonic acid metabolite formed through reactions catalyzed by the cytochrome P450-4A enzymatic complex (CYP4A) in vascular smooth muscle cells and is related to vascular dysfunction and arterial hypertension (**Figure** 10) (76). This molecule has vasoconstrictive action and exerts an important role in vascular function and in the development and progression of cardiovascular diseases (77). Studies have demonstrated the relationship between genetic variations in precursors of 20-HETE formation and the elevation of this metabolite and the BP in humans (78, 79).

In Dahl SS (salt-sensitive) rats, a genetic model of salt-sensitive hypertension, 20-HETE has been shown to contribute to the increase of total peripheral resistance by reducing the ability of the vascular system to respond to direct vasodilation stimulation by reducing vascular function, thus contributing to an increase in BP (80, 81).

Some studies demonstrate that ROS (reactive oxygen species) are important molecules in the development of oxidative stress, playing an important role in vascular dysfunction in Dahl SS rats (82, 83). The chronic exposure to low levels of AngII in these animals may lead to an increase in oxidative stress by elevating ROS cellular concentrations, thus contributing to the reduction of vascular relaxation even when these animals are submitted to a sodium restriction diet or are normotensive (84).

[**FIGURE** 10]

3. MicroRNAs

MicroRNAs (miRNA) are members of a class of small non-coding RNAs that are able to interfere with the translation of several genes (85). They are highly conserved molecules present in many s species and components of the miRNA synthesis machinery can be found in bacteria and species of archaea scattered (86). The miRNAs are single stranded molecules formed of approximately 22 nucleotides, generated from endogenous transcripts (86), expressed from intergenic regions or from introns. The biogenesis of miRNA begins in the nucleus from long precursors called primary miRNAs (pri-miRNA) transcribed by polymerase II (87). The pri-miRNAs, still in the nucleus, are processed by *Drosha* and its cofactor DGCR8 (88, 89), that remove a region of the double-stranded ribbon and flanking sequences resulting the pre-miRNAs. The pre-miRNAs are transported to the nucleus via Exportin-5 (90). In the cytoplasm, the pre-miRNAs are cleaved by a ribonuclease III, called *Dicer*, forming the mature miRNAs (91). The mature miRNA associates to a complex of enzymes called RISC and acts on the translation of mRNA according to the complementarity between the miRNA and its target (92, 93) (**Figure** 11). A complete pairing between the miRNA and its target causes cleavage of the mRNA while an incomplete pairing causes the silencing of the target mRNA expression (94).

The miRNAs are among the most abundant gene regulatory molecules (95) and participate in important regulatory cell pathways, such as apoptosis, cell proliferation, differentiation and development (96-98), and also in various cardiovascular conditions such as cardiac hypertrophy, arrhythmias, apoptosis, and regeneration of cardiomyocytes, fibrosis, heart failure (99), coronary heart disease, acute myocardial infarction (100) and essential hypertension (101).

Besides, miRNAs are currently considered as new hormones or molecules that carry information through intercellular mechanisms. they can be transported by body fluids, such as blood, serum, urine, breast milk, circulating extracellular vesicles, exosomes and microvesicles, in addition to complexes with particles of HDL, protected from degradation by RNAses and other enzymes (102), thus being characterized as potential molecular biomarkers for disease prognosis or as a predictor of therapeutic efficacy (103).

[**FIGURE**11]

3. 1. miRNAs involved in hypertension

Due to the difficulty of studying RH in animal and *in vitro* models, the studies are clinical and poorly understood in terms of mechanisms. Thus, we decided to describe the microRNAs that interact with the most relevant pathways in RH. To date, several miRNAs have been identified and are related to the complications of resistant hypertension (**Figure** 12). The most important are listed below (Table 2):

[TABLE 2]

1. **RAAS**

The miRNAs miR-181a and miR-663 appear to be directly related to the hypertensive condition. Patients with untreated hypertension demonstrated a low expression of miR-181a and miR-663 in kidney samples. Experiments in culture demonstrated that miR-181 and miR-663 were able to regulate renin expression. The data show that the low expression of these two miRNAs causes the elevation of the renin expression leading to the hypertensive condition (104). In addition, the presence of miR-181a in the serum of hypertensive patients was verified. Little is known about the role of miR-181a in the control of renin or whether there are other targets in the kidney. Future work should focus on the regulatory role of miR-181 over the other components of RAAS (105).

The expression of AT_1R (angiotensin II type I receptor) and AT_2R (angiotensin II type 2 receptor) are regulated mainly by post-transcriptional mechanisms. It was verified that miR-155 (expressed in fibroblasts and VSMCs) and miR-802 (expressed in intestinal epithelial cells), in functional assay, are responsible for the repression of AT_1R expression, not by decreasing levels of mRNA, but by blocking mRNA translation. Consequently, it leads to the reduction of activation of Angiotensin II-induced ERK1/ERK2 signaling pathway (106-108). Li et al. demonstrated the existence of a pattern of expression of miRNAs in the plasma of hypertensive patients when compared with controls. In this study, an increase in the expression of miR-296-5p, let-7e, hcmv-miR-UL1 and hcmv-miR-UL112 was verified, which acts via IRF-1 (109). There are evidences that the IRF-1 transcription factor up-regulates the AT_2R and plays a role in angiogenesis, neointima formation, endothelial function and SMC migration (109).

An imbalance in the angiotensin II signaling pathway may lead to hypertensive condition. miR-483-3p is a VSMC-specific miRNA and demonstrates to be a general regulator of RAAS, since several components of this system contain the target sequence for this miRNA. Experiments in cell culture proved that this miRNA acts by suppressing the protein levels of angiotensinogen (AGT), angiotensin I converting enzyme (ACE-1), angiotensin II converting enzyme (ACE-2) and AT_2R without altering the levels of its transcripts (110).

Reports show that miR-132 and miR212 are involved in angiotensin II-induced hypertension. Hypertensive rats show elevated expression of miR-132 and miR-212 in the heart, aortic wall, kidneys and plasma after 10 days of sustained hypertension by angiotensin II infusion. The treatment of hypertensive patients with AT_1R receptor blockers exhibits a lower expression of miR-132 and miR-212 confirming their influence on the hypertension phenotype. miR-132 and miR-212 act by activating the Gαq/ ERK1/ 2 signaling pathway (111). In a case-control study, 9 single nucleotide polymorphisms (SNPs) were genotyped in 7 RAAS-related genes with a common site of miRNA recognition in the 3'-UTR region of the mRNA of these genes. In order to verify the functionality of each SNP in the mRNA/miRNA interaction a dual-luciferase reporter gene was used. Four SNPs located in genes associated with blood pressure were reported: the gene coding the arginine-vasopressin receptor (AVPR1A), the gene coding the bradykinin β2 receptor (BDKRB2), and the gene coding the thromboxane A2 receptor (TBXA2R) (112).

Nossent et al. demonstrated that rs11174811 SNP in AVPR1A interferes in the interaction of this gene with miR-526b and miR -578 and, as a consequence, there is an increase in the expression of vasopressin receptor (112) causing an increase in blood pressure. rs5225 and rs2069591 SNPs in BDKRB2 interfere in the interaction of these genes with miR-34a and miR-34c, culminating in an increase of this receptor expression, consequently inducing vasodilation and inhibiting re-uptake by aquaporin. miR-151-3p inhibits the expression of the bradykinin receptor interacting with the rs2069591 SNP located in the PTK2B gene which, in turn, has its vasopressin expression stimulated. miR-765 interacts with rs13306046 SNP of thromboxane A2 receptor, a potent vasoconstrictor, causing an inhibition of the expression of this receptor and a consequent decrease in blood pressure. miR-383 has been associated with increased risk of myocardial infarction but is not associated with the increase in blood

pressure. This miRNA acts on the rs5534 SNP repressing the NR3C2 gene coding the mineralocorticoid receptor (aldosterone receptor) (112).

In the same way, the polymorphisms encountered in the 3'UTR region of SLC7A1 mRNA (solute carrier family 7 member 1), which has more or less sites for miR-122 binding causing a differential expression of it, leading to hypertension phenotype with endothelial dysfunction with decrease of nitric oxide and L-arginine metabolism phenotype (113-115).

The miRNA 24 is expressed in the adrenal cortex and regulates the production of aldosterone and cortisol, since it binds to the 3'UTR region of the mRNAs CYP11B1 (11β-hydroxylase) and CYP11B2 (aldosterone synthase) which repress them, leading to the decrease in the production of these hormones. Deregulation of production may lead to hypertension. This same miRNA is expressed in cells in the lung where hypoxia leads to the increase of miR-24 expression and the activation of smooth muscle cells (116). Intracellular raise of miR-24 leads to diminished expression of the GATA2 (Endothelial transcription factor GATA2) and PAK4 (Serine/threonine-protein kinase PAK4) transcription factors with functions defined in vascular biology (117) and its inhibition induces the increase of angiogenesis (118).

In experiments with luciferase it was proved that miR-124 and miR-135a are associated with hypertension and they interfere on expression of the nuclear receptor of subfamily 3, group C, member 2 (NR3C2), an aldosterone receptor that participates in the control of blood pressure by promoting the retention of salt by the kidney. miR-124 and miR-135a reduce the protein expression of NR3C2, contributing to the modulation of aldosterone signaling in RAAS, reflecting in the control of blood pressure (119).

miR-584 and miR-31 are predicted to act in a 3'UTR region on 11525C SPN in the human angiotensinogen (AGT) gene. In a cell culture study, the transfection of these miRNAs reduced the levels of AGT expression. Patients that presented 11525A SNP of AGT gene have higher plasma and tissue AGT levels, and consequently increased blood pressure due to the lower interaction of this gene with miR-584 and miR-31 when compared to patients with 11525C SNP (120).

Studies show that miR-145 and miR-143 are essential for differentiation (121) and modulation of vascular smooth muscle cells (VSMC), since the elimination of Dicer

in these cells causes profound hypotension, reflecting *in vivo* contractile dysfunction, blood pressure regulation defects, and vascular remodeling (122). miR-143 and miR-145 demonstrated a relationship to the fate of the VSMCs and maintenance of contractility since KO rats for these two miRNAs exhibit a phenotype that is similar to the rats in which Dicer was eliminated. In addition to this, the overexpression of miR-145 in KO animals for Dicer regains vessel contractility (122).

Boettger et al. demonstrated that the expression of miR-143 and miR-145 during mouse development is essential for the contractile phenotype of VSMC (123). Xin et al. showed that the Serum Response Factor (SRF) controls the expression of miR-143 and miR-145 in VSMC and the absence of these miRNAS causes an imbalance in the feedback cycles needed for the cytoskeleton homeostasis in the VSMC compromising the contractile phenotype of these cells (124). miR-145 acts by suppressing ACE and, in this way, by controlling the stimulation of angiotensin II in VSMCs, thus contributing to the preservation of its contractile phenotype and maintenance of normal blood pressure (123).

miR-143 and miR-145 knockout rats, the actin stress fibers are in disorder, compromising the migratory activity of VSMCs (124), which impair the formation of neointima in response to vascular injury and overregulates (121) the ACE in VSMC (123). There are evidences that these miRNAs are closely involved in the phenotypic reversion of VSMCs during hypertension (121).

In hypertensive patients, miR-9 levels are significantly inferior compared to normotensive patients (125). miR-9 is reported to be a negative regulator of cardiac hypertrophy (126) through inhibition of myocardin. Myocardin is a protein that is found in elevated expression in patients with cardiac hypertrophy and activated by the binding of nuclear factor of activated T-cells c3 (NFATc3) to its promoter region (126).

mir-126 is highly expressed in hypertensive patients (138), which favors angiogenesis and vascular integrity *in vivo* (127, 128). Studies have shown a correlates between miR-126 and Sprouty-related protein (Spred-1) repression (127, 128). Spred-1 interacts and inactivates RAF1 negatively regulating the signaling of the Vascular endothelial growth factor (VEGF) through MAP kinase, by repression of the phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2), a negative regulator of PI3K, another pathway activated by the signaling of the VEGF and with vascular cell adhesion

molecule 1 (VCAM1) repression, limiting the adherence of leucocytes to the endothelial cells (129).

miR-21 is significantly upregulated in hypertension (130) and unlike miR-126, miR-21 acts as a negative modulator of angiogenesis inhibiting the proliferation, migration and the ability to form tubes from endothelial cells and is down-regulated by serum and bFGF. It was observed that the overexpression of miR-21 decreases Ras Homolog Family Member B (RhoB) expression and activity, which appears to be associated with migration and tubulogenesis of endothelial cells (131).

miR-21 is activated by asymmetric dimethylarginine (ADMA), which interferes with angiogenesis by direct inhibition of eNOS, causes dysfunction in the circulation of angiogenic progenitor cells (APCs), represses the expression of superoxide dismutase 2, a key enzyme in the defense of oxidative stress, and inhibit SPRY2, causing MAP kinase ERK activation, resulting in the decrease of NO availability and APC dysfunction (132). After angioplasty, in the vascular wall, there is a significant increase in miR-21 that partially reduces the expression of Phosphatase and tensin homolog **(**PTEN), a phosphatidylinositol 3,4,5-trisphosphate (PIP3) enzyme that inhibits the PI3K pathway by dephosphorylating PIP3 and consequently prevents the activation of AKT (133) and increases the expression of B-cell lymphoma 2 (Bcl-2) (134). Inhibition of miR-21 has a negative effect on neointima formation on vascular injury after angioplasty, decreasing cell proliferation and increasing apoptosis (134).They favor the proliferation of SMCs in culture, inhibiting p27 (Kip1) and p57 (Kip2), which are cell cycle inhibitors, and are upregulated in rat carotid arteries after angioplasty and in smooth muscle cells after vascular wall injury (135, 136). Besides that, it was verified that miR-222 acts by suppressing the expression of miR-221 (135). The treatment of VSMC with platelet-derived growth factor (PDGF) increased miR-221 transcription proving to be critical for the de-differentiation of VSMC, a characteristic of intimal hyperplasia, and caused a decrease in c-kit, a critical gene for the expression of Myocardin (Myocd) and activation of genes in VSMC, modulating the contractile phenotype of these cells. In this study, miR-221 acts via p27 (Kip1) (136).

During aging, there is a progressive increase in the expression of miR-217 in endothelial cells in the same time there is a decrease in the expression of SirT1 (silent information regulator 1), a NAD-dependent deacetylease that controls transcription factors such as FoxO1 (forkhead Transcription factor), p53 and PGC-1a (peroxisome

proliferators activated receptor gamma coactivator-1a). SirT1 is involved in angiogenesis, longevity and resistance to diseases. It was verified that this miRNA represses the expression of SirT1 blocking the angiogenesis. Another miRNA, miR-34a is also related to the control of senescence (137).

miR-29b is the main regulator of several genes related to collagen synthesis besides others related to the production of extracellular matrix which are determinants in renal injury, its suppression causes the increase of the expression of different types of collagens in the renal cortex causing fibrosis of the renal marrow (138). In the heart, the inhibition of miR-29b is associated with cardiac fibrosis and its increased expression inhibits this phenotype. The decreased in the expression of this miRNA is induced by angiotensin II via TGF-β/Smad3 (139).

As exemplified above, miRNAs play a central role in the fine regulation of the RAAS pathway and when expressed in abnormal conditions can cause a number of cardiovascular effects associated with hypertension.

2. Sympathetic

Renal sympathetic activity appears to be essential in maintaining hypertension. Genetically hypertensive mice (BPH/2J) show hyperactivity of the SNS and exacerbate differences in BP during the day and night periods are associated with a greater neuronal activity. Jackson et al. demonstrated that acute sympathetic ganglion blockade with ACE inhibition by enalapril resulted in greater BP declines in BPH/2J animals when compared to normotensive animals, and thus BPH/2J mice presented a sympathetic contribution of up to 4 fold in the elevation of BP. In these animals, renal renin mRNA levels are upregulated and appear to be regulated by miR-181a, which is found to be lower in BPH/2J animals when compared to normotensive animals (140). After bilateral renal denervation, BPH animals show a greater drop in BP after administration of enalapril, lower levels of miR-181a and greater renin expression compared to normotensive animals (141).

Dorr et al. evaluated the effects of renal sympathetic denervation on serum levels of miR-133a. They found elevated levels of miR-133a in the plasma of hypertensive patients 6 months after renal sympathetic denervation demonstrating a correlation between systolic blood pressure and the renal sympathetic nervous system (142).

3. Adipokines

To date, few miRNAS have been associated with hypertension regulation through the adipokine pathway in humans, also correlated with obesity and diabetes. Recently, miR-199a-3p expression has been found to increase during adipocyte cell differentiation and is upregulated in obese individuals. The regulation is mediated by adipokines (leptin, TNF-α, and IL-β increase expression, while resistin decreases expression) in mature adipocytes. This miRNA possesses as possible NLK (nemo-like kinase), VAMP3 (vesicle-associated membrane protein 3) and Cdk7 (cyclin-dependent kinase 7) targets. Thus, it is speculated that this miRNA is related to the modulation in phenotypes of insulin resistance, inflammatory response and diabetes in obese individuals (143).

In a case study with diabetic patients new potential modulators for the adipokine pathway were discovered. Let-7a and let-7f microRNAs were downregulated and miR-326 was upregulated. The latter possesses adiponectin, adiponectin receptor (ADIPOR) -1, ADIPOR-2, and APPL-1 (molecule involved in the intracellular pathway of insulin signaling) as possible targets. In addition, there was an inverse correlation between the concentration of this miRNA and adiponectin. When treated for glycemic control, patients present higher serum levels of let-7a and let-7f microRNAs, but no significant change was observed for miR-326 (144).

In type 2 Diabetes Mellitus, miR-126 is also decreased (145). Zampetaki et al. demonstrated that, in Diabetes Mellitus, elevated glucose levels cause the reduction of miR-126 in apoptotic bodies of endothelial cells, which can cause less delivery of this miRNA, through these apoptotic bodies or microparticles, to the monocytes and in this way increase the resistance to VEGF and contribute to endothelial dysfunction (145).

4. Therapies involving miRNAs

The miRNAs are excellent candidates for therapeutic agents, since that in many diseases, including in hypertension, miRNA levels are altered. This therapeutic tool can be used to re-establish levels of a miRNA and therefore the lost function as demonstrated by Zhang et al. (2014) in which the administration of miR-29b in animal

model of angiotensin II-induced hypertension blocks progressive heart injury demonstrating its function in chronic coronary disease protection (139).

In recent years, the most used strategy is to inhibit inappropriate expression of an altered miRNA (146). For this to happen, chemically modified oligonucleotides, called antagomirs or anti-miRs, were developed which are administered intravenously and are capable of silencing specific endogenous microRNAs (101).

Nevertheless, we still encounter problems regarding the delivery of these oligonucleotides, since a single miRNAs can interact with several RNAs and, depending on the mode of delivery, on different cell types (147). For example, systemic administration of anti-miR and anti-miR-21 blocked the development of cardiac fibrosis but it was also reported to have effects on the development of renal and pulmonary fibrosis (101). To avoid this situation and increase the efficacy of anti-miRs delivery, various delivery modes of such anti-miRs are being tested, though viral carriers such as plasmids, adenovirus, adeno-associated virus, retrovirus and lentivirus, or nonviral vehicles, of which we can highlight the liposomal delivery system, which is a lipid-based nanoparticle (148), such as cholesterol or conjugated to gold nanoparticles, for example (101, 146).

Recently novel classes of miRNA inhibitors are being produced, such as locked-nucleic-acid (LNA), which exhibits greater stability and prolonged storage inside cells by adding a methylene bridge between the 2'-O and the 4'- C atoms in the ribose ring (123); and the artificial sponges of miRNAs, which are produced from transgenes within cells, and bind to miRNA production sites, blocking the transcription of the entire miRNA family (101).

The miRNAs have great potential as biomarkers of innumerous diseases. The miRNAs express a reproducible pattern in biological fluids in hypertensive patients (95, 123) and are easily isolated from plasma, urine, saliva and amniotic fluid (95) without suffering enzymatic degradations and changes due to changes in temperature and pH (102).

[**FIGURE** 12]

CONCLUDING REMARKS AND FUTURE DIRECTIONS

This review aimed to compile the knowledge generated by basic and applied sciences mainly regarding microRNA studies. These may help health professionals improve diagnostic and therapeutic accuracy, reducing mortality and morbidity in RH.

Epigenetic as well as genetic factors are identified every day and they are associated with variation in blood pressure levels. As reviewed herein, mutation-polymorphism in some signaling pathway gene may increase or decrease the expression of some microRNAs which are involved both in RH development, therapy response as RH associated complications such as renal failure, coronary artery disease, cardiac hypertrophy, stroke among others. Therefore, the use of microRNA as biomarkers in prevention, diagnosis and therapy of this disease, may help to understand the disease, improve pharmacology therapy as well prevent complications.

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Figure Legends

FIGURE 1: Recommendations for Diagnostics of RH. ABPM: Ambulatory Blood Pressure Monitoring; BP: Blood Pressure; RH: Resistant Hypertension; WCH: White-Coat Hypertension. Modified Calhoun (1) and Passarelli, Gonçalves (16)

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FIGURE 11: Biogenesis of miRNAs in mammals. 1. miRNAs are transcripted in the nucleus by RNA polymerase II from long precursors. 2. In the nucleus they are processed by a complex formed by Drosha and its cofactor DGCR8. 3. 3. The pre-miRNAs are transported to the cytoplasm by the Exportin-5 receptor. 4. In the cytoplasm, the pre-miRNAs are processed by Dicer resulting in the formation of miRNA:miRNA duplex 5. The miRNA:miRNA duplex is processed by helicase 6. Association with RISC complex. 7. Repression or inhibition of mRNA.

FIGURE 12. Scheme of miRNA pathway in Hypertension. Scheme of miRNA pathway in Hypertension. Major microRNAs involved in the hypertension pathogenesis. In red, those that act on muscle cells; In green, those presents on cardiomyocytes; In blue, those that are expressed in endothelial cells; In brown, those that act on fibroblasts; In purple, present in renal cells; in yellow, those that are expressed in adipocytes**;** In black, the present in plasma. NO – Nitric Oxide; ERK – extracellular regulated kinase, GATA2 - Endothelial transcription factor GATA2; PAK4 - Serine/threonine-protein kinase PAK4; CYP11B1 - 11β-hydroxylase CYP11B2 - aldosterone synthase; Sprouty-related protein (Spred-1); Vascular Smooth Muscle Cells (VSMCs); C-X-C motif ligand 12 - CXCL12; nuclear receptor of subfamily 3 group C member 2 - NR3C2; monocyte chemoattractant protein-1 -MCP-1; Angiotensin type 2 receptor - AT2R; Cyclin-dependent kinase inhibitor 1B - p27, Kip1; Cyclin-dependent kinase inhibitor 1C -p57, Kip2; Vascular Endothelial Growth Factor (VEGF); Vascular Cell Adhesion Molecule 1 (VCAM-1); Ras Homolog Family Member B (RhoB), Phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2); Phosphatase and tensin homolog (PTEN); Protein sprouty homolog 2 (Sprouty-2); Angiotensin-Converting Enzyme (ACE); ; Silent information regulator 1 (SirT1); Angiotensin II Receptor (AT1R); Angiotensinogen (AGT); arginine-vasopressin receptor (AVPR1A); bradykinin β2 receptor (BDKRB2); thromboxane A2 receptor (TBXA2R); B-cell lymphoma 2 (Bcl-2)

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FIGURE 9: EGFR Signaling Pathway in vascular smooth muscle. The agonist action of the GPCR receptors stimulates NOX1 phatway lead to the increase of the oxidative stress NOX1which the activating the signaling pathway of the EGFR. – NADPH oxidase 1; ROS – Reactive oxygen species; MMP – Matrix Metalloproteinase; HB-EGF – Heparin biding-Epidermal growth factor; EGFR – Epidermal growth factor receptor; GPCR – G protein coupled receptor; PLC – Phospholipase C; DAG – Diacylglycerol; IP3 – Inositol triphosphate; PKC – Protein kinase C; PI3K – Phosphoinositide 3 kinase; PIP2 – Phosphatidylinositol 4,5-biphosphate; PIP3 – Phosphatidylinositol (3,4,5)-triphosphate; AKT – Protein kinase B; mTOR – Mammalian target of rapamycin.

FIGURE 10: Ang II stimulates signaling pathway through 20-HETE. The stimulate of GPCR by AngII agonist activate PLA2 which shoots the signaling intracellular phathway leading activation of PKC as release calcium influx. ROS – Reactive oxygen species; GPCR – G protein coupled receptor; PLA2 – Phospholipase A2; CYP4A – Cytochrome P450-4A; COX – Cyclooxygenase; LO – Lipoxygenase ; LTs – Leukotrienes; 20-HETE – 20-hydroxyeicosatetraenoic acid; PGE2 – Prostaglandin E2; PGI2 – Prostacyclin; PGH2 – Prostaglandin H2; TXA2 – Tromboxane A2; MAPK – Mitogen-activated protein kinase; NO – Nitric oxide;

FIGURE 11: Biogenesis of miRNAs in mammals. 1. miRNAs are transcripted in the nucleus by RNA polymerase II from long precursors. 2. In the nucleus they are processed by a complex formed by Drosha and its cofactor DGCR8. 3. 3. The pre-miRNAs are transported to the cytoplasm by the Exportin-5 receptor. 4. In the cytoplasm, the pre-miRNAs are processed by Dicer resulting in the formation of miRNA:miRNA duplex 5. The miRNA:miRNA duplex is processed by helicase 6. Association with RISC complex. 7. Repression or inhibition of mRNA.

FIGURE 12. Scheme of miRNA pathway in Hypertension. Scheme of miRNA pathway in Hypertension. Major microRNAs involved in the hypertension pathogenesis. In red, those that act on muscle cells; In green, those presents on cardiomyocytes; In blue, those that are expressed in endothelial cells; In brown, those that act on fibroblasts; In purple, present in renal cells; in yellow, those that are expressed in adipocytes**;** In black, the present in plasma. NO – Nitric Oxide; ERK – extracellular regulated kinase, GATA2 - Endothelial transcription factor GATA2; PAK4 - Serine/threonine-protein kinase PAK4; CYP11B1 - 11β-hydroxylase CYP11B2 - aldosterone synthase; Sprouty-related protein (Spred-1); Vascular Smooth Muscle Cells (VSMCs); C-X-C motif ligand 12 - CXCL12; nuclear receptor of subfamily 3 group C member 2 - NR3C2; monocyte chemoattractant protein-1 -MCP-1; Angiotensin type 2 receptor - AT2R; Cyclin-dependent kinase inhibitor 1B - p27, Kip1; Cyclin-dependent kinase inhibitor 1C -p57, Kip2; Vascular Endothelial Growth Factor (VEGF); Vascular Cell Adhesion Molecule 1 (VCAM-1); Ras Homolog Family Member B (RhoB), Phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2); Phosphatase and tensin homolog (PTEN); Protein sprouty homolog 2 (Sprouty-2); Angiotensin-Converting Enzyme (ACE); ; Silent information regulator 1 (SirT1); Angiotensin II Receptor (AT1R); Angiotensinogen (AGT); arginine-vasopressin receptor (AVPR1A); bradykinin β2 receptor (BDKRB2); thromboxane A2 receptor (TBXA2R); B-cell lymphoma 2 (Bcl-2)

TABLE 1: Forms of Secondary Hypertension Associated with RH. Modified Vongpatanasin (14)

Conditions	Obstructive Sleep Apnea	Primary Aldoteronism	Renal Artery Stenosis	Renal Parenchyma Disease	Use of drugs and alcohol	Thyroid Disorders
Diagnostic Tests	Polysomnography	Serum aldosterone, plasma renin activity	Duplex Doppler ultrasonography, Computedtomographic angiography, or magnetic resonance angiography	Serum creatinine	History taking	Thyrotropin, free thyroxine
Treatment	Continuous positive airway Pressure	Spironolactone, eplerenone, orsurgical resection of tumor inunilateral aldosterone-producing Adenoma	Renal revascularization in selected patients	Correction of underlying causes if possible	Discontinuation of offendingagents	According to underlying disorders
Prevalence in RH (%)	60-70	7-20	2-24	1-2	2-4	<1
References	(7, 149, 150)	(8, 9, 151-154)	(7, 155-157)	(1, 7)	(1, 7, 158)	(1, 7)

Table 2. miRNAs related to hypertension

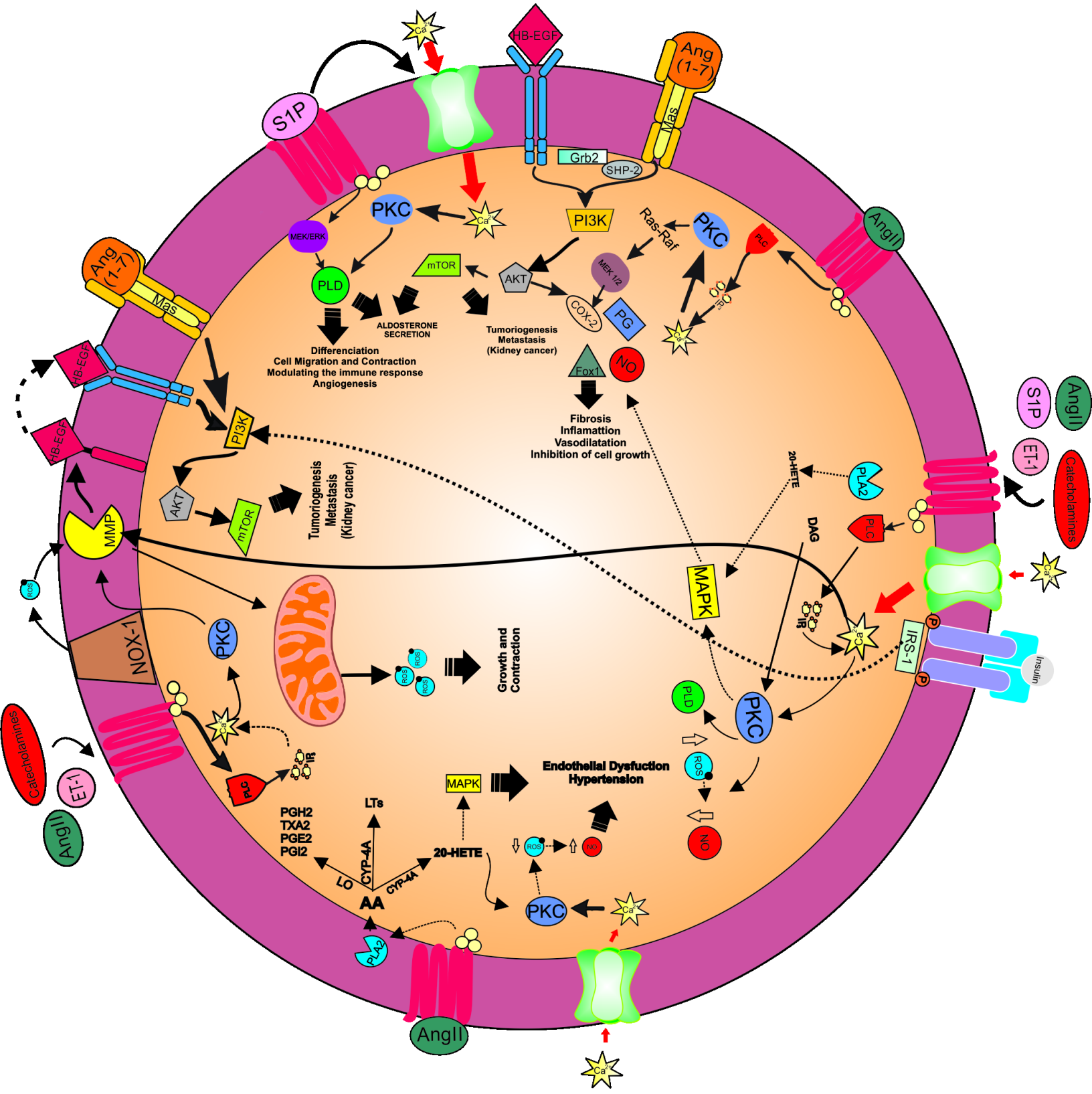
MiRNA	Cells Type	Target	References
miR-126	Endothelial apoptotic bodies Endothelial cells Endothelial apoptotic bodies Plasma Zebrafish embryos Endothelial cells Peripheral blood mononuclear cells Mouse Endothelial cells	VEGF VCAM-1 SPRED-1 and PIK3R2	(127-129, 138, 145)
miR-21	Endothelial cells Angiogenic progenitor cells Rat vascular smooth muscle cells Murine hearts Vascular smooth muscle cells	RhoB Superoxide dismutase 2 Sprouty-2 PTEN Bcl-2	(130-134)
miR-143 and miR-145	Mice/Rat/Mouse VSMCs	ACE Modulators of SRF activity	(121-124)
miR-221 and miR-222	Rat VSMCs	p27 (Kip1) p57 (Kip2) c-Kit	(135-136)
miR-217	Human umbilical vein endothelial cells Human aortic endothelial cells	SirT1	(137)

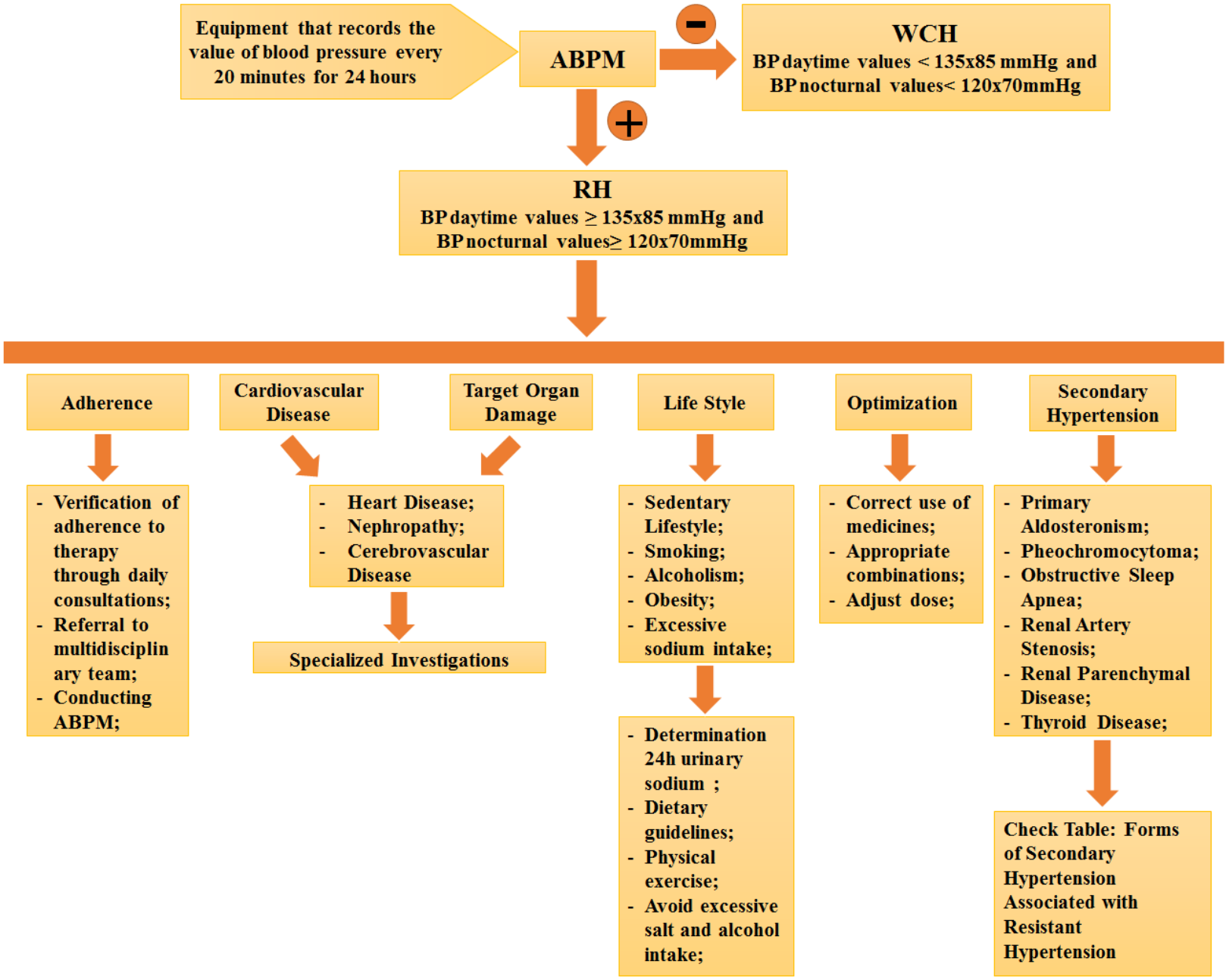
	Human coronary artery endothelial cells		
miR-9	Rats Cardiomyocytes PBMCs	Myocardin	(125-126)
miR-155 and miR-802	CHO cells C2BBe1 cell line Rat aortic adventitial fibroblasts	AT_1R	(106-108)
miR-296-5p, let-7e, hcmv-miR-UL112 and hcmv-miR-UL1	Plasma	IRF-1 MICB	(109)
miR-483-3p	HEK-AT_1R HEK-AT_2R HASMC HL1-AT_1R RASMC RASMC-AT_1R RASMC-AT_2R Whole heart (C3H NT) Whole heart (C3H TG) Whole heart (C57BL/6 NT) Whole heart (C57BL/6 TG)	AGT ECA-1 ECA2 AT_2R	(110)
miR-122	Plasma Mouse Endothelial Cells	rs41318021 polymorphism in the SLC7A1 gene 3´UTR of the L-Arginine Transporter Gene	(113-115)

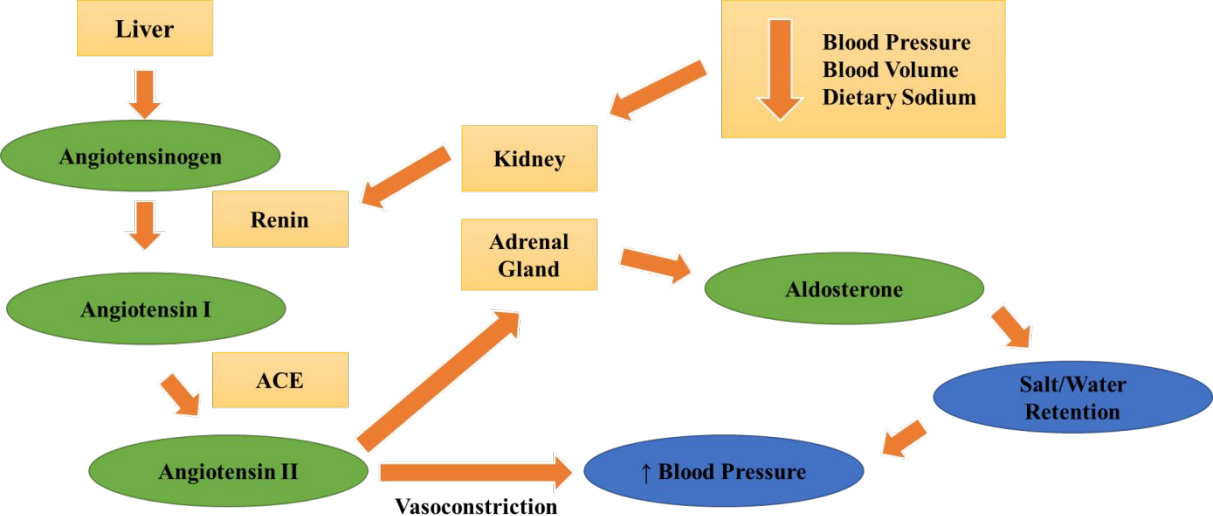
		SLC7A1	
miR-24	Cardiac endothelial cells Mouse Endothelial cells, cardiomyocytes and cardiac fibroblasts HUVECs and HMVECs Human adrenocortical cell line	GATA2 PAK4 CYP11B1 CYP11B2	(116-118)
miR-124 and miR-135a	Study *In silico*	Mineralocorticoid receptor *NR3C2*	(119)
miR-526b, miR-578, miR-34a, miR-34c, miR-765 mir-153-3p and miR-383	Study of Myocardial Infarctions Leiden	Four SNPs located in the arginine vasopressin 1A receptor, bradykinin 2 receptor and thromboxane A2 receptor	(112)
miR-584 and miR-31	Hep3B cells HEK293 cells Genetically hypertensive mice (BPH/2J)	Polymorphism at 11525C in hAGT 3′-UTR	(120, 140, 141)
miR-181a and miR-663	HEK293 cells Plasma	Renin	(104-105)
miR-29b	Mouse heart Cardiac fibroblastos Rat renal medullary epithelial cells	TGF-β1 Collagen genes	(138-139)
miR-132 and miR-212	Rat heart, aorta and kidney Rat Plasma	AT1R	(111)

	Human artery		
miR-199a-3p	Adipocytes	NLK VAMP3 Cdk7	(143)
miR-326	Plasm	ADIPOR-1 ADIPOR-2 APPL-1	(144)
miR-133a	Plasm	-	(142)

Sprouty-related protein (Spred-1); Vascular Smooth Muscle Cells (VSMCs); Vascular Endothelial Growth Factor (VEGF); Vascular Cell Adhesion Molecule 1 (VCAM-1); Phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2); Phosphatase and tensin homolog (PTEN); Ras Homolog Family Member B (RhoB); Protein sprouty homolog 2 (Sprouty-2); Angiotensin-Converting Enzyme (ACE); Serum Response Factor (SRF); Silent information regulator 1 (SirT1); Angiotensin II Receptor (AT_1R); Transforming Growth Factor (TGF)-β1; Angiotensinogen (AGT); nemo-like kinase (NLK); vesicle associated membrane protein 3 (VAMP3); cyclin-dependent kinase 7 (Cdk7); adiponectin receptor 1(ADIPOR-1); adiponectin receptor 2 (ADIPOR-2); Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL-1)


S1P
HB-EGF
Ang (1-7)
Mas
Grb2
SHP-2
PKC
PI3K
Ras-Raf
MEK/ERK
PLD
mTOR
AKT
MEK 1/2
COX-2
PG
Fox1
NO
PLC
AngII
ALDOSTERONE SECRETION
Differenciation
Cell Migration and Contraction
Modulating the immune response
Angiogenesis
Tumoriogenesis
Metastasis
(Kidney cancer)
Fibrosis
Inflamattion
Vasodilatation
Inhibition of cell growth
MMP
ROS
NOX-1
MAPK
20-HETE
PLA2
DAG
IP3
IRS-1
Insulin
Growth and Contraction
Endothelial Dysfuction
Hypertension
PGH2
TXA2
PGE2
PGI2
LTs
LO
CYP-4A
AA
Catecholamines
ET-1


Equipment that records the value of blood pressure every 20 minutes for 24 hours
ABPM
WCH
BP daytime values < 135x85 mmHg and BP nocturnal values< 120x70mmHg
RH
BP daytime values ≥ 135x85 mmHg and BP nocturnal values≥ 120x70mmHg
Adherence
- Verification of adherence to therapy through daily consultations;
- Referral to multidisciplinary team;
- Conducting ABPM;
Cardiovascular Disease
Target Organ Damage
- Heart Disease;
- Nephropathy;
- Cerebrovascular Disease
Specialized Investigations
Life Style
- Sedentary Lifestyle;
- Smoking;
- Alcoholism;
- Obesity;
- Excessive sodium intake;
- Determination 24h urinary sodium ;
- Dietary guidelines;
- Physical exercise;
- Avoid excessive salt and alcohol intake;
Optimization
- Correct use of medicines;
- Appropriate combinations;
- Adjust dose;
Secondary Hypertension
- Primary Aldosteronism;
- Pheochromocytoma;
- Obstructive Sleep Apnea;
- Renal Artery Stenosis;
- Renal Parenchymal Disease;
- Thyroid Disease;
Check Table: Forms of Secondary Hypertension Associated with Resistant Hypertension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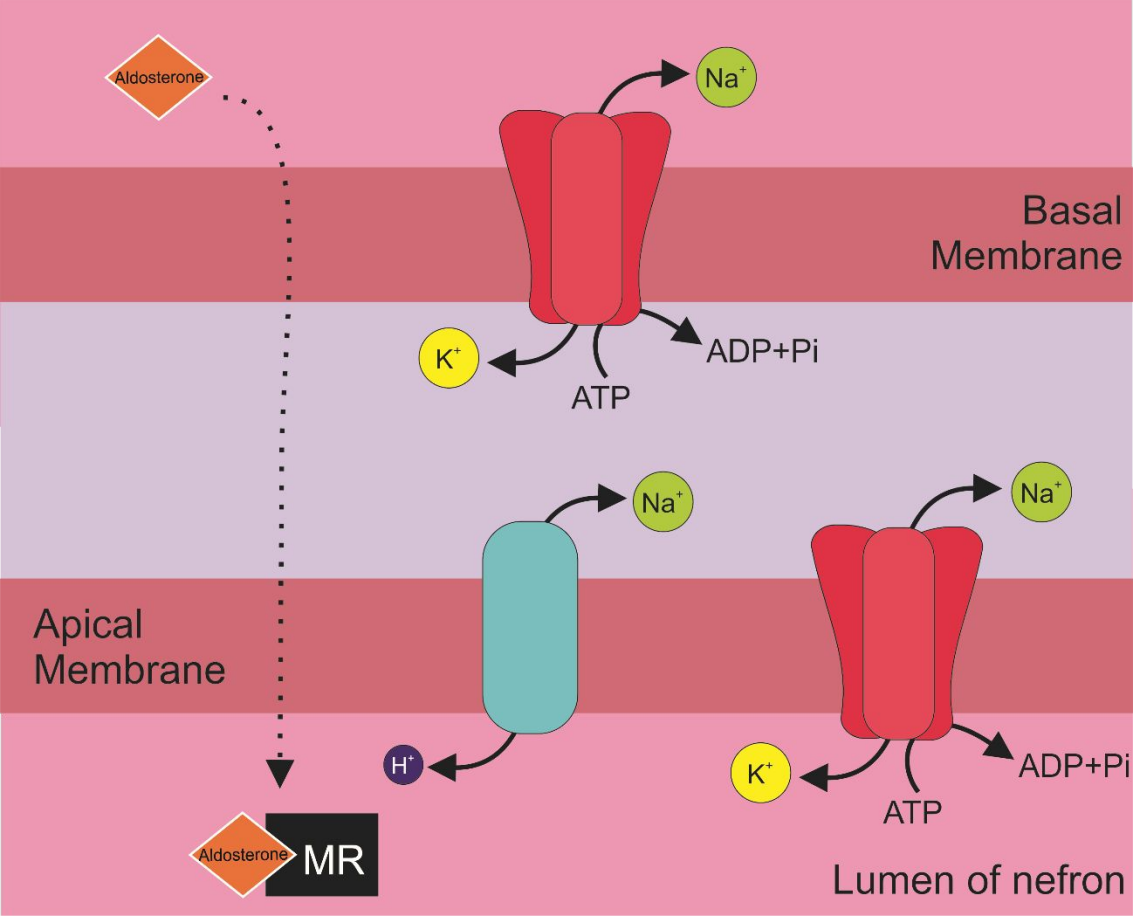
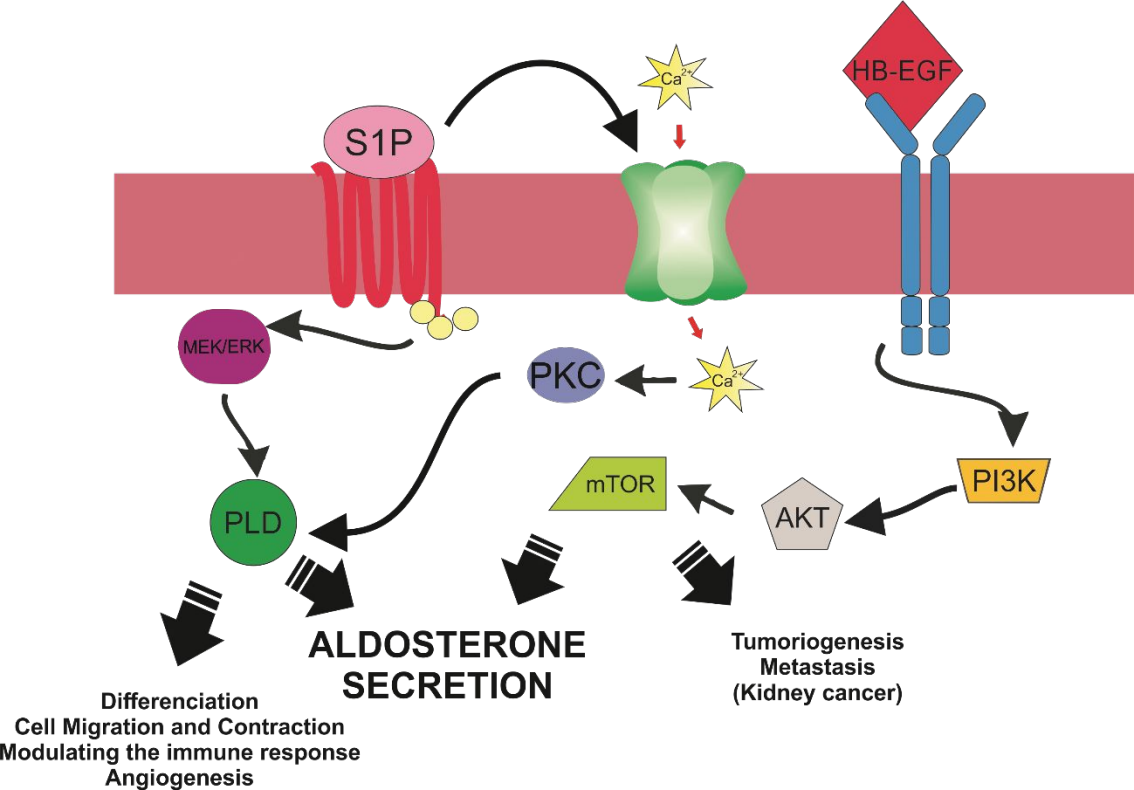
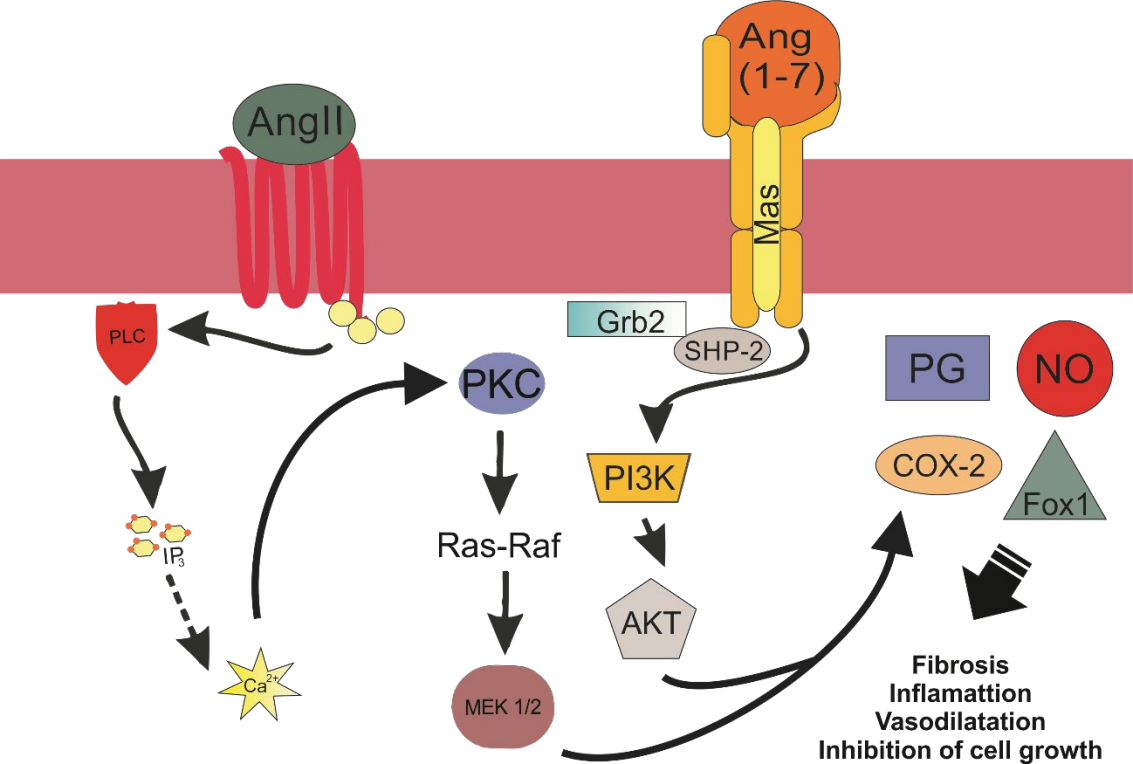
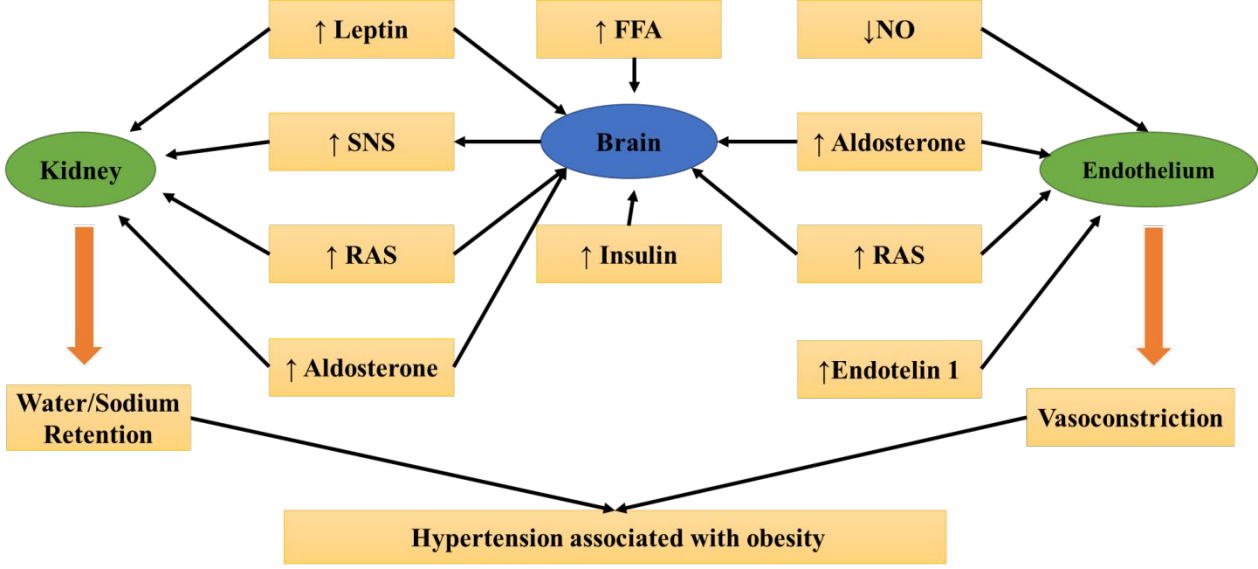
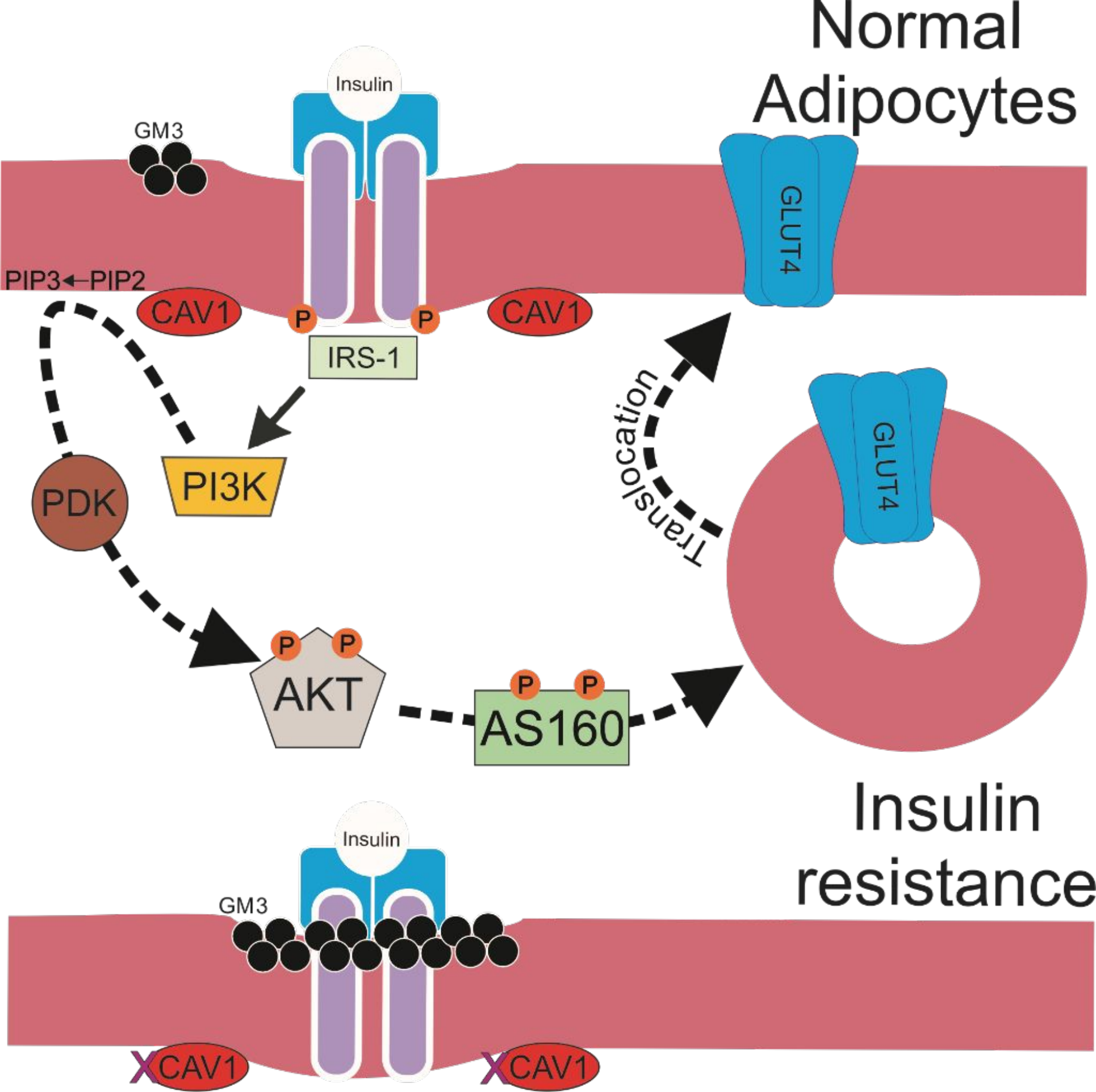
Blood Pressure
Blood Volume
Dietary Sodium
Angiotensinogen
Kidney
Renin
Adrenal Gland
Aldosterone
Angiotensin I
ACE
Salt/Water Retention
Angiotensin II
↑ Blood Pressure
Vasoconstriction

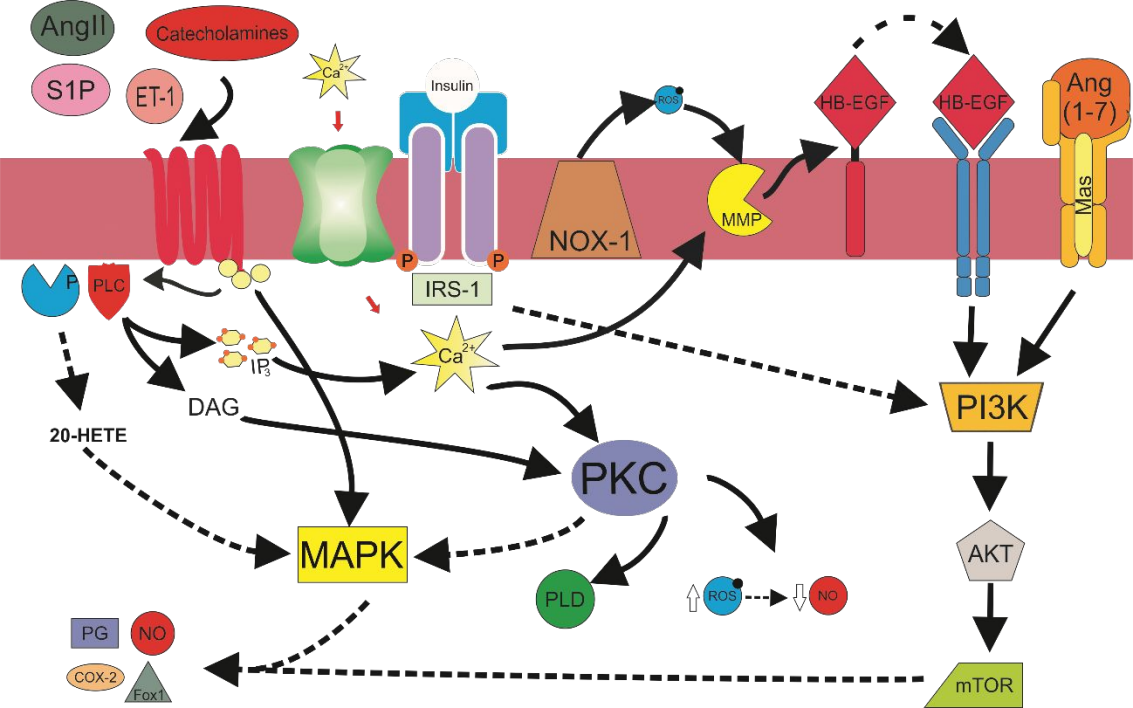

Aldosterone
Na+
Basal Membrane
K+
ATP
ADP+Pi
Na+
Apical Membrane
H+
Na+
K+
ATP
ADP+Pi
Aldosterone
MR
Lumen of nefron

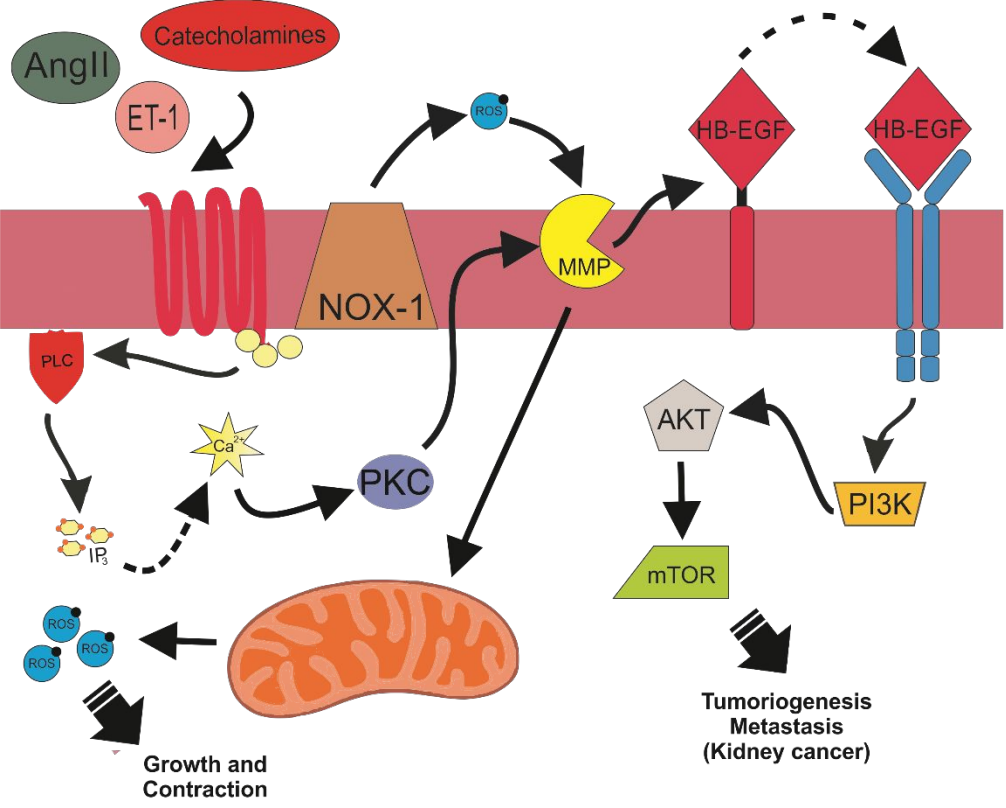

S1P
Ca2+
HB-EGF
MEK/ERK
PKC
Ca2+
PI3K
mTOR
AKT
PLD
ALDOSTERONE SECRETION
Tumoriogenesis
Metastasis
(Kidney cancer)
Differenciation
Cell Migration and Contraction
Modulating the immune response
Angiogenesis


AngII
Ang
(1-7)
Mas
PLC
Grb2
SHP-2
PKC
PG
NO
PI3K
COX-2
Fox1
IP3
Ras-Raf
AKT
Ca2+
MEK 1/2
Fibrosis
Inflamattion
Vasodilatation
Inhibition of cell growth


↑ Leptin
↑ FFA
↓NO
Kidney
↑ SNS
Brain
↑ Aldosterone
Endothelium
↑ RAS
↑ Insulin
↑ RAS
↑ Aldosterone
↑Endotelin 1
Water/Sodium Retention
Vasoconstriction
Hypertension associated with obesity


Normal Adipocytes
Insulin
GM3
GLUT4
PIP3←PIP2
CAV1
P
P
CAV1
IRS-1
Translocation
GLUT4
PDK
PI3K
P
P
AKT
P
P
AS160
Insulin resistance
Insulin
GM3
XCAV1
XCAV1


AngII
Catecholamines
S1P
ET-1
Ca2+
Insulin
ROS
HB-EGF
HB-EGF
Ang (1-7)
Mas
NOX-1
MMP
P
P
IRS-1
PLC
IP3
Ca2+
DAG
20-HETE
PI3K
PKC
MAPK
AKT
PLD
ROS
NO
PG
NO
COX-2
Fox1
mTOR


AngII
Catecholamines
ET-1
ROS
HB-EGF
HB-EGF
NOX-1
MMP
PLC
AKT
Ca2+
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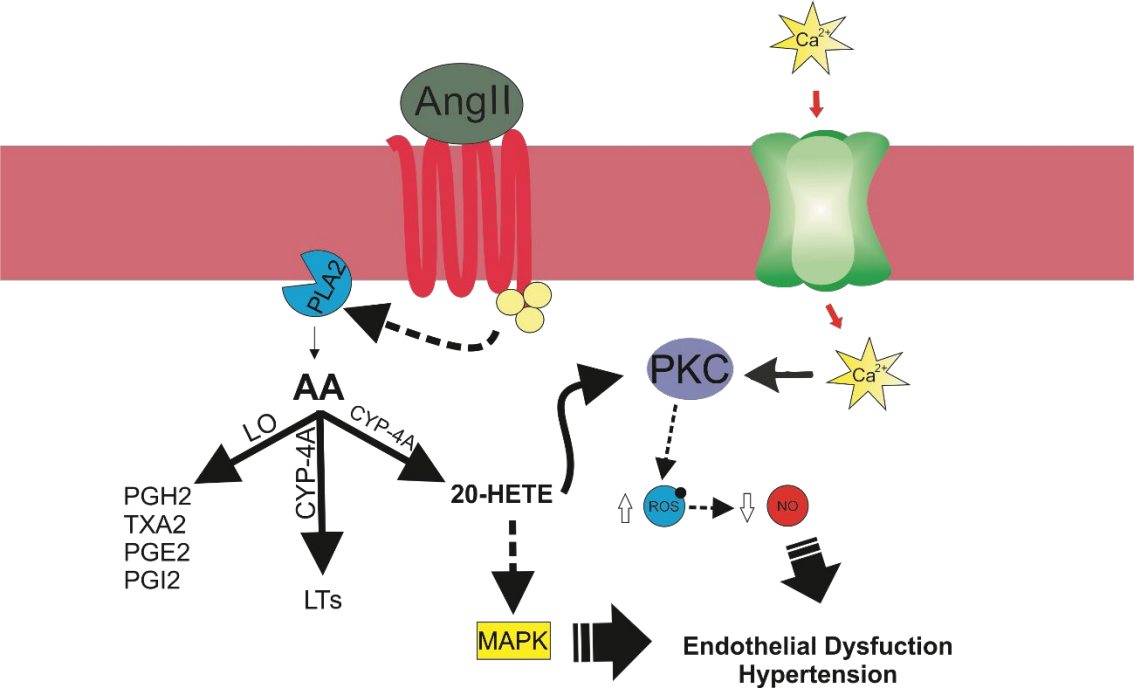
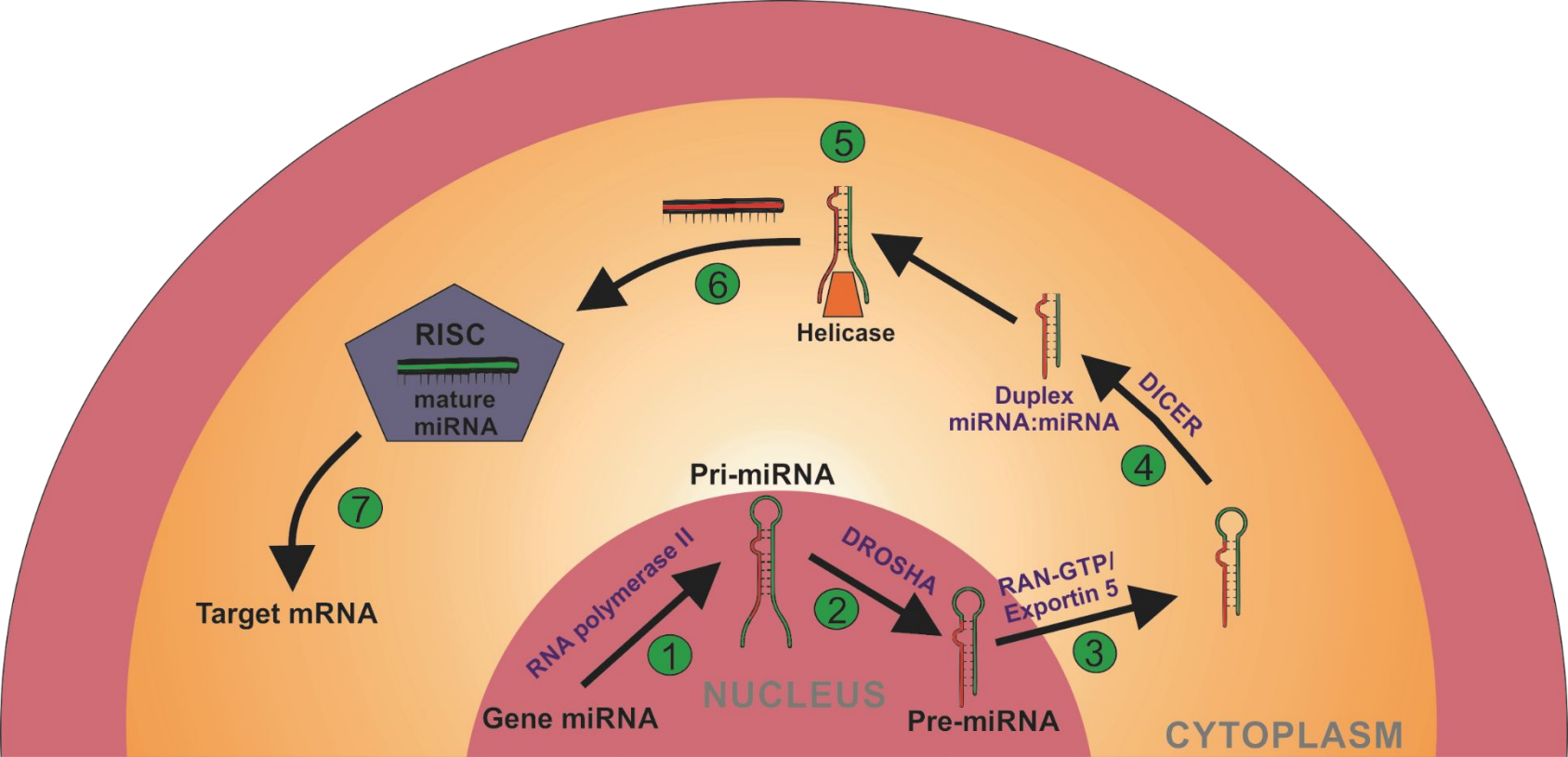
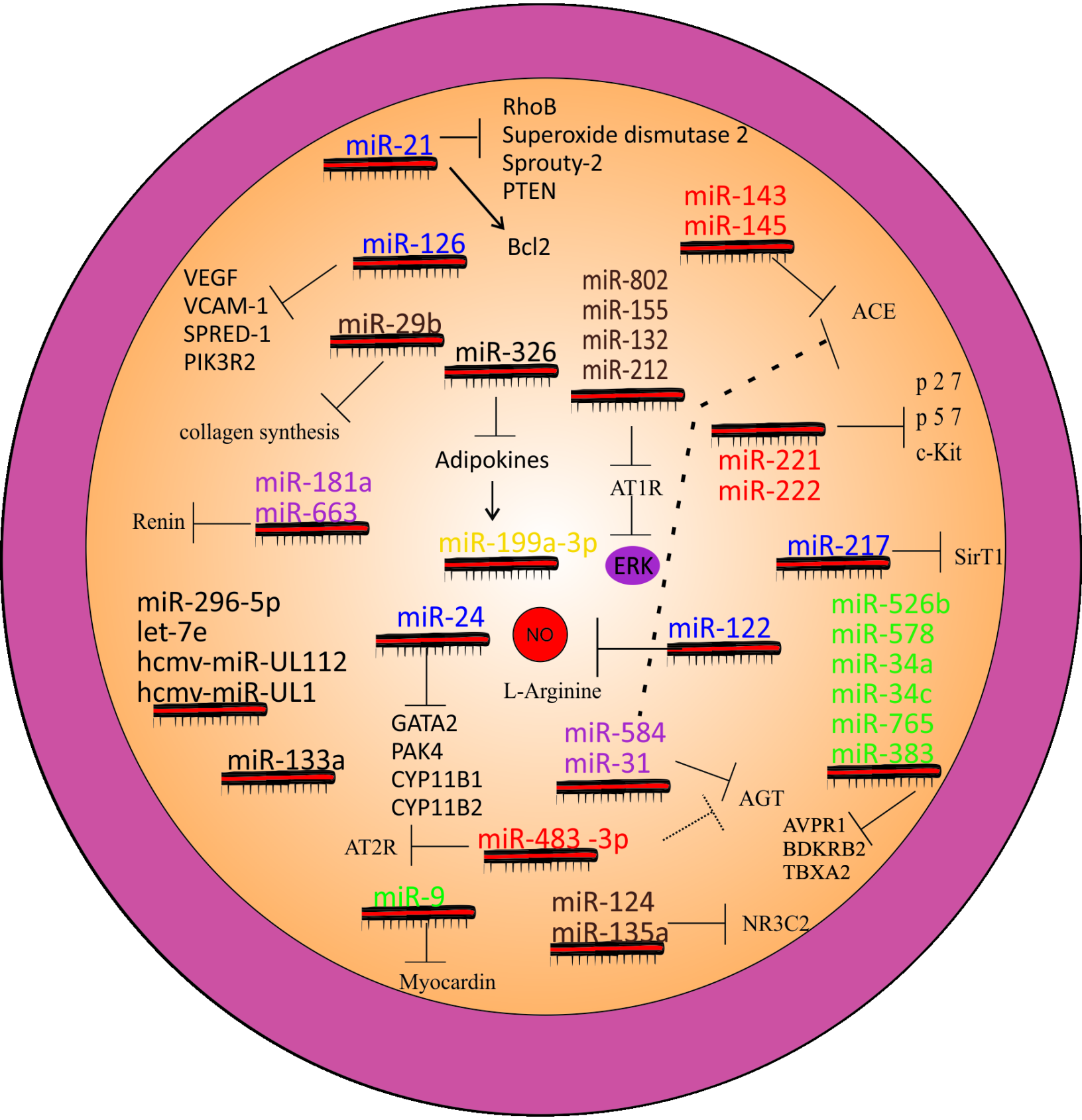
PI3K
IP3
mTOR
ROS
ROS
ROS
Tumoriogenesis
Metastasis
(Kidney cancer)
Growth and
Contraction


Ca2+
AngII
PLA2
AA
LO
CYP-4A
CYP-4A
PGH2
TXA2
PGE2
PGI2
LTs
20-HETE
PKC
Ca2+
ROS
NO
MAPK
Endothelial Dysfuction
Hypertension


5
6
RISC
mature
miRNA
Helicase
Duplex
miRNA:miRNA
DICER
4
7
Target mRNA
Pri-miRNA
RNA polymerase II
DROSHA
RAN-GTP/
Exportin 5
1
2
3
Gene miRNA
NUCLEUS
Pre-miRNA
CYTOPLASM


miR-21
RhoB
Superoxide dismutase 2
Sprouty-2
PTEN
Bcl2
miR-126
VEGF
VCAM-1
SPRED-1
PIK3R2
miR-29b
collagen synthesis
miR-326
Adipokines
miR-143
miR-145
ACE
miR-802
miR-155
miR-132
miR-212
AT1R
p 2 7
p 5 7
c-Kit
miR-221
miR-222
miR-181a
miR-663
Renin
miR-199a-3p
ERK
miR-217
SirT1
miR-296-5p
let-7e
hcmv-miR-UL112
hcmv-miR-UL1
miR-24
NO
L-Arginine
miR-122
miR-526b
miR-578
miR-34a
miR-34c
miR-765
miR-383
GATA2
PAK4
CYP11B1
CYP11B2
miR-133a
miR-584
miR-31
AGT
AVPR1
BDKRB2
TBXA2
AT2R
miR-483 -3p
miR-9
Myocardin
miR-124
miR-135a
NR3C2